SOME EXPERIMENTS BEARING UPON DROPLET INFECTION IN DIPHTHERIA.*

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The occurrence of cases of laryngeal diphtheria without involvement of the pharynx is strong evidence in favor of the view that infection sometimes occurs through inhalation of diphtheria bacilli. If infection is by inhalation in these cases, then it seems not unlikely that a certain percentage of the pharyngeal cases are also infected in this manner; for floating particles containing viable diphtheria bacilli may be caught upon the mucous membrane of the pharynx as readily as upon that of the larynx and there is no indication that the latter is more susceptible to infection than the former.

It is conceivable that viable diphtheria bacilli may be inhaled either contained within fine droplets of saliva or attached to particles of dust. This investigation was undertaken with the idea of obtaining a more definite conception than we possess of the part played by the former of these methods in the spread of diphtheria.

That gross particles of saliva and even pieces of membrane are occasionally thrown out by diphtheria patients in coughing is well known, and the danger of infection to one immediately in front of the patient is obvious; but infection in this manner is probably of rare occurrence and is not considered in this paper. The possibility of infection through the inhalation of particles of dust containing diphtheria bacilli also will not be discussed.

The importance of droplet infection in the spread of diphtheria will depend primarily upon the number of droplets containing viable diphtheria bacilli that are emitted by the patients, but not upon this factor alone.

The possible influence of another factor is illustrated in the incidence of epidemics of pneumonic plague. This disease spread with amazing rapidity during the epidemic in Manchuria in the

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winter of 1910–11. On the other hand, though there have been numerous isolated cases of plague pneumonia in India (probably more than 1 per cent of all the plague cases), yet this form of the disease has not assumed epidemic proportions there. It seems probable that droplets containing plague bacilli were thrown out in large numbers in both instances, but that in the hot climate of India the plague bacilli in the droplets quickly suffered death from drying, whereas, in the bitterly cold climate of Manchuria, with its poorly heated dwellings, the bacilli remained alive in the air for a relatively long period of time. This explanation of the occurrence of epidemics of pneumonic plague in cold climates was recently advanced and experimentally supported by Teague and Barber.[1]

The second factor bearing upon the importance of droplet infection in diphtheria is, then, the length of time that the diphtheria bacilli contained in the floating droplets remain alive; the danger of infection is obviously the greater, the longer the living diphtheria bacilli remain suspended in the air in the neighborhood of the patient. This factor will depend primarily upon the resistance of diphtheria bacilli to death from drying and to a less extent upon the condition of the atmosphere with regard to temperature, moisture, and light in the individual case.

I first attempted to determine whether the patients in coughing and talking emit diphtheria bacilli frequently, or only rarely, and whether in large numbers, or only in small numbers. Extensive experiments have been carried out by Flügge, his pupils, and others with reference to the emission of tubercle bacilli, but I have been unable to find any experimental data in the literature with regard to the emission of diphtheria bacilli.

MODE OF PROCEDURE.

Loeffler's serum medium was used exclusively. The mixture of ox-serum and glucose broth was solidified in petri dishes about 4 inches in diameter and then sterilized by two further heatings in the Arnold sterilizer. Only freshly prepared plates were used and care was taken that the surface of the medium should not be too dry. Just before the plates were used, the excess of water

[1] *Philippine Jour. Sc.*, 1912 7, p. 157.

was removed with a pipette and then either the plates were kept partially uncovered in the incubator for about half an hour, or circles of sterile blotting paper were applied to the surface of the medium for a few minutes and then removed.

Usually plates were exposed before two or three different cases on each visit to the hospital. The patient was, first of all, requested to expectorate into a sterile empty petri dish and a little of the saliva thus obtained was inoculated upon one of the Loeffler plates by means of a sterile swab. The patient was then requested to talk (to repeat the alphabet, count to 50, and so forth) while an open Loeffler plate was held about three inches from his or her mouth. These two procedures, namely, the culture of the saliva and the exposure of a plate during talking, were always carried out before the patient was asked to cough and before cultures were made from the secretions on the tonsils. The tonsillar cultures were also made upon Loeffler plates in order to obtain a better idea of the number of diphtheria colonies than is afforded by tube cultures. Plates were held in like manner about three inches from the mouth and the patient was told to cough a number of times. If the very young patients did not cough spontaneously, a spatula was sometimes touched against the pharynx to excite coughing; in other instances the child was held lying on its back in bed and the plates were exposed before its mouth while it cried.

In less than an hour and a half after their exposure before the patients, the plates were placed in the incubator. They were examined daily for four or five days, smears from the separate colonies being stained with Loeffler's methylene blue solution. Any colony that contained diphtheria bacilli was recorded as a diphtheria colony, even though there was an abundance of other organisms and only a few diphtheria bacilli present in the stained smear; in other words, mixed colonies containing diphtheria bacilli are included among the diphtheria colonies, in the table. However colonies which apparently contained only diphtheria bacilli were much more frequently met with than these mixed colonies.

For the recognition of *B. diphtheriae* the morphology of the bacillus when stained with Loeffler's methylene blue solution was

alone relied upon. It was not deemed necessary for the purposes of this investigation to carry out fermentation tests or animal inoculations. Therefore a few errors may have crept in, since certain colonies containing diphtheroids may have been recorded in the diphtheria column and, on the other hand, a few colonies containing diphtheria bacilli may have been overlooked; but such errors could not have affected the results materially.

A few of the exposed plates were overgrown with liquefying organisms; such plates were discarded.

In the first 23 cases, cultures of the saliva were not made.

The first plates were exposed on October 9, 1912, and the last on January 4, 1913. The exposures were all made in the well-heated and well-ventilated wards of the Willard Parker Hospital. All of the patients had received diphtheria antitoxin.

Plates were exposed before 54 cases, but those of cases No. 30, No. 39, and No. 40 were not examined. The remaining 51 cases represent 49 different patients, since cases No. 8 and No. 28 were the same patients as No. 5 and No. 24 respectively, plates having been exposed before these two patients both while the tube was in the larynx and after its removal. Of the 49 patients, 29 were males and 20 were females.

In 40 of the 51 cases, cultures of the secretions upon the tonsils were positive and three other cases may be regarded as certainly positive; namely, patient No. 17, who was dying of clinical diphtheria, for which reason the tonsillar culture was not made; patient No. 34, whose tonsillar plate was overgrown by a liquefying organism, but the saliva was found to be positive; and case No. 6, from which diphtheria colonies were found on the exposed plates. Of the eight negative cases, one (No. 28) had been found positive by me four days before, two (No. 38 and No. 50) had been reported positive by the Bureau of Health a few days previous to my examination, and three (No. 19, No. 23, and No. 45) were croup cases. The control cultures of case No. 31 were overgrown with a liquefying organism. The remaining negative case was No. 21. It seems not unlikely, therefore, that diphtheria bacilli were present in small numbers in the throat or larynx of most of these negative cases at the time the plates were exposed before them.

TABLE 1.

Case Number	Age in Years	Days Ill	Membrane Visible	Control Cultures		Plates Exposed	Results		Remarks
				Saliva	Tonsil		Colonies	Diphtheria Colonies	
1	4	6	No	..	+++	3 coughs and crying	10	1	
						3 coughs	5	0	
						Speaking about 20 words	16	0	
2	8	..	No	..	+	Speaking about 80 words	3	0	
						4 coughs	5	0	
						4 coughs	12	1	
3	7(?)	5	No		+++	Speaking about 60 words	8	0	
						6 coughs	6	0	
						9 coughs	9	0	
4	3½	4	Yes	..	+++	Speaking 12 words	11	7	All of the diphtheria colonies in a single cluster
						Crying	5	0	
						4 coughs and crying	13	0	
5	5	8	Yes	..	++	4 attempts at coughing	1	1	Intubated 3 days ago. Tube still in larynx.
						6 attempts at coughing	6	0	
6	2½	4	No	..	Negative	Loud crying for about 1 min	15	1	
						Loud crying for about 1 min	19	0	
						7 coughs	19	1	
7	18	10	No	..	+	Counting 50 and repeating alphabet	32	0	
						5 coughs	75	1	
						5 coughs	16	0	
8 (Same patient as No. 5)	5	10	Yes	..	++	8 coughs	19	0	Intubated 5 days ago. Extubated a few hours ago.
						8 coughs through 4 layers of gauze	13	0	
						14 coughs	14	0	
						10 coughs through 2 layers of gauze	45	0	
						10 coughs	18	0	
						10 coughs through 1 layer of gauze	15	0	
9	25	6	Yes	..	+++	Counting to 30 and repeating alphabet	12	0	
						4 coughs	20	0	
						7 coughs	39	0	
						9 coughs through 2 layers of gauze	14	0	
10	26	3	Yes	..	++	Counting to 50	13	0	
						6 coughs	36	0	
						10 coughs	18	0	

TABLE 1—*Continued.*

Case Number	Age in Years	Days Ill	Membrane Visible	Control Cultures		Plates Exposed	Results		Remarks
				Saliva	Tonsil		Colonies	Diphtheria Colonies	
11	3⅓	4	..	..	+++	7 attempts at coughing 11 attempts at coughing	15 19	1 1	Intubated a few hours ago.
12	2½	6	..	..	++	Attempts at crying Attempts at crying Attempts at crying	7 32 23	1 0 0	Intubated 4 days ago. Coughed up tube today. Voiceless.
13	7	4	Yes	..	+++	One grunt and repeating the alphabet... Counting to 50 Counting to 50	35 9 18	4 0 1	
14	3	5	..	..	+	5 coughs and talking 3 coughs 4 coughs	22 12 3	0 3 0	Intubated 2 days ago. Tube still in larynx.
15	2½	..	..	..	+	Crying, nostrils compressed Crying, nostrils compressed Crying	3 7 3	0 0 0	Extubated 2 days ago.
16	5	3	Yes	..	+++	Counting to 50 5 coughs 6 coughs	8 7 3	0 0 0	
17	4	3(?)	..	..	..	Breathing about 20 times Breathing about 20 times	4 0	0 0	Intubated today. Moribund.
18	7(?)	7	..	..	+	3 coughs 4 spontaneous coughs	6 8	0 0	Extubated this morning.
19	4	9	..	..	Negative	3 natural coughs 10 coughs 7 coughs	About 300 3 7	0 0 0	Extubated this morning.
20	5	6	Yes	..	+++	Counting to 40 3 coughs 6 coughs	5 5 19	0 1 1	
21	6	2	Yes	..	Negative	Crying and 2 coughs, spatula in mouth.. Counting to 20 2 attempts at coughing	120 10 12	0 0 0	

TABLE 1—*Continued.*

Case Number	Age in Years	Days Ill	Membrane Visible	Control Cultures		Plates Exposed	Results		Remarks
				Saliva	Tonsil		Colonies	Diphtheria Colonies	
22	7	..	No	..	++	Counting to 50 5 weak coughs	63 18	0 0	Carrier for 12 days.
23	1⅛	5	No	..	Negative	3 coughs 6 coughs 5 coughs 6 coughs 12 coughs	18 8 28 6 8	0 0 0 0 0	Croup. Not intubated.
24	4½	8	..	++	+++	1 attempt at coughing 2 attempts at coughing	9 17	0 0	Intubated yesterday.
25	4(?)	5	..	Negative	+	5 attempts at coughing Talking and attempts at coughing 7 attempts at coughing	11 22 31	1 0 1	Intubated 4 days ago. Tube still in larynx.
26	24	5	Yes	+++	+++	Repeating alphabet and counting 100 by fives twice 10 coughs 7 coughs Talking 3 loud coughs	15 48 12 28 19	0 8 5 1 4	Coughed up cast of trachea just before plates were exposed.
27	4	4	..	..	++	1 cough with spatula in mouth 5 good coughs 3 coughs	11 12 About 150	1 0 0	Intubated today.
28 (Same patient as No. 24)	4½	12	..	Negative	Negative	2 weak coughs 3 natural coughs 3 natural coughs	1 4 0	0 0 0	Intubated 5 days ago. Extubated today. (Control cultures positive 4 days ago.)
29	19	6	Yes	++	+++	Counting to 50 10 loud coughs 5 loud coughs Singing about 30 words 12 loud coughs 8 coughs 7 coughs	1 5 3 2 4 4 15	0 3 0 0 2 0 0	

TABLE 1—*Continued.*

Case Number	Age in Years	Days Ill	Membrane Visible	Control Cultures		Plates Exposed	Results		Remarks
				Saliva	Tonsil		Colonies	Diphtheria Colonies	
31	7	4	Yes	Negative	Negative	Counting to 50	8	0	Control cultures overgrown with a liquefying organism.
						Singing about 40 words	4	0	
32	37	7	No	Negative	+	4 strong coughs	18	0	
						4 loud coughs	14	0	
						Counting to 50	22	0	
33	36	4	Yes	Negative	+++	10 forcible coughs	25	1	
						4 coughs	11	0	
						Counting to 50	5	0	
						Counting to 50 in a low voice	10	0	
34	18	4	Yes	++	Plate liquefied	7 good coughs	30	1	
						Counting to 50	15	0	
35	8	3	Yes	+++	+++	Talking	10	0	
						6 weak coughs	20	0	
						5 weak coughs	6	0	
36	4½	..	Yes	++	+++	Coughing twice and crying	50	2	
						Crying	6	0	
						Crying	2	0	
						Talking	7	0	
37	34	5	Yes	+++	+++	6 loud coughs	20	0	
						Counting to 50 slowly, distinctly	23	0	
						Speaking about 20 words	20	1	
						Speaking about 30 words	2	0	
						4 coughs	0	0	
						5 coughs	0	0	
38	28	4	No	Negative	Negative	9 loud coughs	60	0	
						3 loud coughs	0	0	
41	5	5	..	+	++	Whispering and counting to 50	10	1	Intubated 2 days ago. Tube still in larynx.
						8 half-spontaneous coughs	4	0	
						8 natural coughs	6	0	
						12 coughs	5	0	

TABLE 1—*Continued.*

Case Number	Age in Years	Days Ill	Membrane Visible	Control Cultures		Plates Exposed	Results		Remarks
				Saliva	Tonsil		Colonies	Diphtheria Colonies	
42	9	5	Yes	++	++	Counting to 100 and repeating alphabet	15	0	
						6 attempts at coughing	24	0	
						12 attempts at coughing (a small piece of membrane thrown upon plate)	53	23	
						15 attempts at coughing	325	47	
43	16	4	Yes	++	+++	Counting to 50, repeating alphabet and Lord's prayer	10	0	
						5 attempts at coughing	4	1	
						6 attempts at coughing	8	1	
						10 coughs	42	4	
44	30	3	Yes	+	++	Counting to 50 and talking	16	0	
						Singing about 50 words	15	0	
						8 weak attempts at coughing	6	0	
						12 attempts at coughing	24	1	
45	5	2(?)	No	Negative	Negative	Hoarse crying	3	0	Croup. Not intubated.
						Hoarse crying	8	0	
						5 coughs	24	0	
						10 coughs	13	0	
46	4⅔	5	..	+	++	Hoarse crying	42	15	Croup. Not intubated.
						Hoarse crying	18	0	
						Hoarse crying	36	1	
						Hoarse crying	20	0	
47	2½	4	No	++	++	Heavy breathing	22	0	Intubated 2 days ago. Tube still in larynx.
						3 weak coughs, spatula in mouth	1	0	
						4 weak coughs, spatula in mouth	2	0	
						2 weak coughs, spatula in mouth	2	0	
48	6	7	?	+	++	Counting to 30 and talking	11	2	Intubated this morning.
						2 weak attempts at coughing	16	5	
						7 coughs after drinking water	28	6	
						8 attempts at coughing	4	0	

TABLE 1—*Continued.*

Case Number	Age in Years	Days Ill	Membrane Visible	Control Cultures		Plates Exposed	Results		Remarks
				Saliva	Tonsil		Colonies	Diphtheria Colonies	
49	8	5	Yes	++	+++	Counting to 50 and repeating alphabet..	4	1	
						13 attempts at coughing.....	2	1	
						9 good coughs.....	13	1	
						10 good coughs.....	11	1	
						7 good coughs.....	6	1	
						5 good coughs.....	2	0	
50	24	5	..	Negative	Negative	Counting to 50 and repeating alphabet..	4	0	Culture reported positive 2 days ago.
						5 forcible coughs.....	34	0	
						5 forcible coughs.....	18	0	
						7 forcible coughs.....	80	0	
						9 coughs.....	75	0	
51	2	4	Yes	++	+++	Loud crying for 3 min.....	3	0	
						Loud crying for 2 min.....	1	0	
						Loud crying for 1 min.....	1	0	
						Loud crying for 2 min.....	1	0	
52	27	4	Yes	Negative	+	Counting to 30 and talking.....	4	0	
						15 attempts at coughing.....	29	0	
						8 weak attempts at coughing.....	7	0	
						6 attempts at coughing.....	26	0	
						18 weak attempts at coughing.....	2	0	
53	19	5	Yes	++	++	Counting to 50 and alphabet.....	1	0	
						6 coughs.....	14	0	
						10 coughs.....	24	1	
						4 coughs.....	9	0	
54	11	3	Yes	++	+++	Counting to 50, repeating alphabet and talking.....	2	0	
						12 coughs, some natural.....	8	0	
						6 strong attempts at coughing.....	4	0	
						10 coughs.....	8	4	
						12 coughs.....	15	6	

+++=confluent growth of diphtheria bacilli.
++=numerous scattered diphtheria colonies.
+=a few diphtheria colonies.

To summarize the results given in the preceding table: of the 51 cases, 28 were proved to have thrown droplets containing viable diphtheria bacilli upon at least one of the plates exposed before them. Of 180 plates exposed before the patients, 48 were positive for diphtheria bacilli. In other words, more than half of the cases emitted diphtheria bacilli in coughing or talking and more than one-fourth of the plates exposed showed diphtheria colonies. If the eight cases, in which the control cultures were negative, are omitted, then 65 per cent of the cases emitted diphtheria bacilli and 31 per cent of the exposed plates were positive. When it is borne in mind that the plates were exposed before the patients only for very short periods (from a few seconds to two or three minutes), that many of the attempts at coughing were less forcible than spontaneous coughs, and finally that only a fraction of the emitted droplets were caught upon the plates, these results are very striking. For the sake of clearness the results are summarized in tabular form.

TABLE 2.

	All Cases	All Cases Except Those with Negative Control Cultures*	Cases in Which Control Cultures Contained Numerous Diphtheria Colonies†
Total number of cases	51	43	34
Number of cases that emitted diphtheria bacilli	28	28	23
Number of cases that did not emit diphtheria bacilli	23	15	11
Percentage of cases that emitted diphtheria bacilli	55	65	68
Total number of plates exposed	180	153	124
Number containing diphtheria colonies	48	48	41
Number containing no diphtheria colonies	132	105	83
Percentage of positive plates	27	31	33
Average number of diphtheria colonies on positive plates	3.75	3.75	4.2

* Cases Nos. 19, 21, 23, 28, 31, 38, 45, and 50 omitted.

† Cases Nos. 2, 6, 7, 14, 15, 17, 25, 32, and 52 omitted in addition to the eight cases with negative cultures.

Thirty of the 48 positive plates contained only a single diphtheria colony each. Only three of the plates showed more than eight diphtheria colonies, and two of these were exposed under rather artificial conditions (the patient grunting and blowing instead of coughing) and may, therefore, be disregarded.

Four of the cases will be described in somewhat more detail than in the table.

Case 26.—A man, 24 years old, had been ill 5 days. Diphtheritic membranes on the uvula and both tonsils. Just before my arrival at the hospital, the patient had coughed up a tubular cast of the trachea about three and a half inches long. Cultures from the tonsil and from the blood-stained sputum showed a confluent growth of diphtheria. The patient sat up in bed with assistance and talked in a fairly loud voice. The coughs were loud and strong. At each cough I could feel the expired air striking forcibly against the exposed plate. Diphtheria colonies developed upon four of the five plates exposed, but the largest number of diphtheria colonies upon a plate was only eight.

Case 29.—A man, 19 years old, had been ill 6 days. An extensive diphtheritic membrane on the posterior wall of the pharynx and uvula. The patient was strong enough to sit up in bed without assistance and talked in a loud conversational tone. Good coughing, a strong current of air striking the plate with each cough. The culture from the throat showed a confluent growth of diphtheria, that of the saliva about 150 scattered diphtheria colonies upon the plate. Seven plates were exposed, two of which showed two and three diphtheria colonies respectively.

Case 42.—A boy, 9 years old, had been ill 5 days. He had apparently had an extensive membrane, though I did not determine to what extent the membrane was still present. Throat badly swollen. The patient sat up in bed and made earnest and forcible attempts at coughing but did not actually cough. His attempts consisted largely in grunting and blowing. The conditions were, therefore, decidedly artificial and no great importance can be attached to the results. Large numbers of diphtheria colonies developed upon two of the four plates exposed.

A small piece of membrane was thrown upon one of the plates. This was the only instance in the course of these experiments in which a gross particle was seen to strike the plate.

Case 51.—A two-year-old male baby that had been ill 4 days. An extensive diphtheritic membrane covered the posterior pharynx and the tonsils. The control culture from the tonsils showed a confluent growth of diphtheria. A little saliva swabbed from beneath the tip of the patient's tongue brought about the development of about 75 scattered diphtheria colonies on a Loeffler plate. The baby was held lying on its back in bed by the nurse and plates were exposed before its mouth, for 1 minute, 2 minutes, and 3 minutes respectively, while it cried in a loud voice. No diphtheria colonies and very few other colonies developed on the plates.

These experiments indicate that droplets containing viable diphtheria bacilli are emitted very frequently by diphtheria patients in talking and coughing, but usually in small numbers only.

Since coughing consists in subjecting the air behind the closed vocal cords to high pressure and then suddenly opening the cords, it was thought that perhaps laryngeal cases would emit vastly more droplets containing diphtheria bacilli than pharyngeal or tonsillar cases. If this were true, obviously the laryngeal cases

should receive special attention with regard to isolation, nursing, etc. It was found, however, that the laryngeal cases investigated did not emit appreciably more droplets containing *B. diphtheriae* than did the other cases. But since many of the laryngeal cases had been intubated and hence could not cough in the manner indicated above, it was decided to attempt to throw further light upon this question by carrying out experiments with *B. prodigiosus*.

It was planned to determine whether more droplets containing *B. prodigiosus* were emitted on coughing and talking after swabbing the larynx with a suspension of this bacillus than after swabbing the pharynx and tonsils or the mouth with the same suspension. Dr. Wells P. Eagleton kindly agreed to swab my larynx. Agar plates, upon which *B. prodigiosus* produced red colonies, were exposed about two or three inches from the mouth during coughing and talking. "Now is the time for all good men to come to the aid of their party," a sentence of 16 words, containing most of the letters of the alphabet, was selected for the short exposures during talking, and the Lord's Prayer, the alphabet, and counting to 50 (about 150 words) for the longer ones.

TABLE 3.

	After Swabbing Larynx	After Swabbing Uvula, Tonsils, and Pillars	After Swabbing Teeth, Lips, Tongue, and Palate
	Prodigiosus Colonies	Prodigiosus Colonies	Prodigiosus Colonies
16 words	0	0	0
2 coughs	13	3	11
150 words	0	0	5
5 coughs	4	2	32
Control	0	0	0
16 words	0	0	1
2 coughs	0	1	11
150 words	0	0	2
5 coughs	0	1	4
Control	0	0	0

The greater part of one agar slant of *B. prodigiosus* was suspended in about 10 c.c. of 0.8 per cent sodium chlorid solution. The larynx was swabbed with this suspension, care being taken not to contaminate the mouth or pharynx during the procedure. Plates were then exposed during coughing and talking. Control plates were held before the mouth during quiet breathing. After

that the uvula, tonsils, and pillars were swabbed with the same suspension and plates were exposed in the same manner as before. Finally the teeth, lips, tongue, and palate were swabbed with the suspension of *B. prodigiosus* and plates were exposed in like manner for the third time. The result of this experiment is given in Table 3.

It is seen from Table 3 that no colonies of *B. prodigiosus* developed on the plates exposed during talking and only a few colonies on those exposed during coughing after the larynx had been swabbed. In this experiment much larger amounts of the suspension were used in swabbing the pharynx and mouth than in swabbing the larynx. For this reason further similar experiments were performed, approximately the same amount of the suspension having been used at each swabbing. Dr. J. L. Dias kindly applied the prodigiosus suspension and Dr. J. J. Smith and Dr. E. W. Sprague acted as subjects.

TABLE 4.

	Dr. Sprague as Subject	Dr. Smith as Subject		Dr. Sprague as Subject	Dr. Smith as Subject
	Prodigiosus Colonies	Prodigiosus Colonies		Prodigiosus Colonies	Prodigiosus Colonies
Larynx Swabbed:			Tonsils, Uvula, and Pillars Reswabbed:		
16 words	0	4	2 coughs	2	7
150 words	0	15	5 coughs	48	25
16 words	0	1	2 coughs	275	3
150 words	0	7	5 coughs	117	6
Larynx Reswabbed:			Tongue, Lips, and Mucosa of Cheeks Swabbed:		
2 coughs	6	53	16 words	3	2
5 coughs	34	14	150 words	11	19
2 coughs	1	2	16 words	5	2
5 coughs	2	2	150 words	20	23
Tonsils, Uvula, and Pillars Swabbed:			Tongue, Lips, and Mucosa of Cheeks Reswabbed:		
16 words	0	0	2 coughs	10	2
150 words	2	3	5 coughs	8	10
16 words	0	0	2 coughs	7	13
150 words	0	2	5 coughs	1	12

The results shown in Table 4 agree with those of the first experiment. These experiments all indicate that in talking and coughing no more droplets are thrown out from the larynx than from the

mouth and that if a larger series of suitable cases of laryngeal diphtheria had been investigated, the results would not have been materially different from those obtained.

It was shown by Hutchison[1] that *B. prodigiosus* contained in fine droplets may be carried 53 meters along a corridor and up two flights of stairs; in another experiment he found that it was carried by a breeze over as great a distance as 600 meters. Other investigators have obtained similar results.

Since *B. diphtheriae* is much more resistant to death from drying than *B. prodigiosus*, we may assume that, under similar circumstances, it would be carried over even greater distances.

Many investigators have shown that *B. diphtheriae* is very resistant to drying under various conditions, but there are few experiments indicating the relative resistance of *B. diphtheriae* and *B. prodigiosus*. Kirstein[2] sprayed suspensions of different bacteria and allowed only the finer droplets to settle upon empty sterile petri dishes, which were then kept at room temperature. Culture medium was added to the plates at various intervals of time and the number of developing colonies recorded. He sums up his results as follows:

Kind of Bacteria	Average Time of Death of Bacteria after Spraying
B. prodigiosus	24 hrs.
B. typhosus	24 hrs.
B. diphtheriae	24–48 hrs.
Staphylococcus aureus	8–10 days

The following experiment, which I performed, demonstrates more strikingly the greater resistance of *B. diphtheriae*. Suspensions of the different bacteria to be tested were prepared in 0.8 per cent sodium chlorid solution and made of approximately the same degree of cloudiness as judged by the eye. Bits of sterilized absorbent cotton were dipped into the suspensions, thoroughly squeezed out between the thumb and finger, and quickly rubbed over the surface of about a dozen carefully cleaned and sterilized slides. The slides lying upon a piece of blotting paper, the surface of which had been sterilized in the free flame, were then placed in an incubator at 37° C. At intervals slides were removed and placed face down upon agar plates; after an hour they were moved back and forth across the surface of the agar and then removed. The plates were incubated for several days and the developing colonies counted with the results shown on p. 413.

The vast number of experiments in the literature upon the resistance of bacteria to drying affords a rather confused picture, because they were performed under such widely different conditions. It would seem to be highly desirable to classify the bacteria into groups with reference to their resistance to drying by the use of some such method as that just described.

[1] *Ztschr. f. Hyg. u. Infectionskrankh.*, 1901, 36, p. 223.

[2] *Ztschr. f. Hyg. u. Infectionskrankh.*, *1902*, *39*, p. *163*.

Interval	Cholera	Prodigiosus	Typhoid	Diphtheria	Staphylococcus
At once (control)....	Colonies innumerable	Colonies innumerable	Colonies innumerable	Colonies innumerable	Colonies innumerable
2 min.....	0	..	..	..	..
½ hr.....	..	140	..	..	..
1½ hrs.....	..	60	..	..	..
2 hrs.....	..	..	Numerous	Numerous	Numerous
3 hrs.....	..	2	..	..	..
4 hrs.....	..	6	..	..	..
9½ hrs.....	..	2	125	64	..
20 hrs.....	..	1	9	28	..
28 hrs.....	..	0	14	25	..
36 hrs.....	..	..	6	40	600
48 hrs.....	..	0	1	4	406
60 hrs.....	..	..	0	3	..
3 days.....	..	..	0	0	130
4 days.....	..	..	1	1	94
5 days.....	..	..	1	0	102
6 days.....	..	..	..	..	147
7 days.....	..	..	..	..	41

Working in the tropics, Dr. Barber and the writer found that, after being sprayed, *B. prodigiosus* disappeared from the air more rapidly than previous experiments carried out in temperate climates would lead one to expect. By spraying in an atmosphere saturated with water vapor, we showed that this disappearance of living prodigiosus bacilli from the air was due to death from drying and not to settling. We also sprayed *B. prodigiosus* in a cold storage room at a temperature of 12° C., and were the first to point out the marked effect of the low temperature in prolonging the life of the bacilli in suspended droplets. This last observation has, without doubt, an important bearing upon droplet infection in general. An individual with influenza must be far more dangerous in a cold room than in a warm one; for we know that the influenza bacillus is readily killed by drying and the longer the bacilli remain alive in the air, obviously the greater the danger of infection. It seems not unlikely that the increased danger of infection is in part responsible for what is often spoken of as the "lowered resistance" of the individual from remaining in an insufficiently heated room. Also, one meets with the argument that droplet infection plays no part in certain diseases, because medical students and others visit hospitals containing patients with these diseases and escape infection. This argument is fallacious for the reason that a well-heated and well-ventilated hospital ward offers different conditions with regard to the persistence of droplets in the air from what

would be met with, for example, in a poorly heated tenement house in a cold climate.

CONCLUSIONS.

In talking and coughing, diphtheria patients frequently emit droplets containing viable diphtheria bacilli, but they emit such droplets usually in small numbers only.

Patients with laryngeal diphtheria apparently do not throw out many more droplets containing diphtheria bacilli than do pharyngeal cases. In agreement with this observation, it was found that after swabbing the larynx with a suspension of *B. prodigiosus*, no more droplets containing this bacillus are thrown out in coughing and talking than after swabbing the pharynx and mouth.

The diphtheria bacillus is much more resistant to death from drying than *B. prodigiosus*. Hence, the former would tend to remain alive in suspended droplets longer than does *B. prodigiosus* in the numerous series of experiments in which it has been used.

It is pointed out that bacteria in suspended droplets would remain alive much longer in poorly heated tenements during cold weather than is indicated by laboratory experiments performed at higher temperatures.

It is believed that the data furnished by these experiments will be of aid to the epidemiologist and to the sanitarian in arriving at a correct estimate of the importance of the part played by droplet infection, as compared to other modes of infection, in causing the spread of diphtheria.

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